Patent
Attorney's Docket No. 028723-063

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	)	**MAIL STOP AF**
Rolf J. MEHLHORN	)	Group Art Unit: 1615
Application No.: 08/472,843	)	Examiner: G. Kishore
Filed: June 7, 1995	)	Confirmation No.: 1044
For: METHOD FOR LOADING LIPID LIKE VESICLES WITH DRUGS OR OTHER CHEMICALS	)	

DECLARATION OF ERIC G. MAYHEW, PH.D.

Commissioner for Patents
PO Box 1450
Alexandria, Virginia 22313-1450

Sir:

I, Eric G. Mayhew, Ph.D., hereby declare and state:

1. I am currently a private consultant to Celator Inc., Vancouver, BC and NeoPharm Inc., Chicago, IL, as well as owner of MayPharm Consulting Services, WA. I have a B.Sc. in Biology/Chemistry (1960), an M.Sc. (1963) and a Ph.D. (1967) in Cell Biology, and a D.Sc. (1993) from the University of London, England. I was a principal scientist at the Liposome Company, Princeton, NJ from 1993-1999, and a consultant therefore from 1999-2000. A copy of my *curriculum vitae* is attached.

2. I have been asked to review the claims of the above-identified application, as well as the Office Action dated December 20, 2002 and the references cited in the Office Action, and to give my opinion as to whether these references disclose or suggest the subject matter of the amended claims submitted concurrently with this Declaration.

3. I have reviewed the Office Action dated December 20, 2002, as well as the cited references of Deamer et al., Cramer et al., and Kano et al. I do not agree with the Examiner that any of these references, alone or in combination, disclose or suggest the invention which is currently claimed in the amended claims submitted concurrently with this Declaration.

4. Deamer et al. used atebrine and 9-aminoacridine as fluorescent probes for measuring pH gradients across membranes. Deamer et al. uses liposomes to analyze the quenching effect on these fluorescent probes in the presence of a pH gradient. Deamer et al. does not disclose or suggest loading liposomes with drugs to make pharmaceutical preparations for administration to animals; nor does Deamer et al. disclose or suggest suspending the vesicles for administration in a bulk solution, wherein the bulk solution has a pH which is physiologically benign, as is now required by independent Claims 46 and 52.

5. In fact, Deamer et al. teaches away from suspending the vesicles for administration in a bulk solution having a pH which is physiologically benign (i.e., about pH 7.0-7.4). Deamer et al. analyzes the movement of a fluorescent probe into liposomes (fluorescence enhancement) at pH 5-9. Deamer et al. states at p. 326 that "the enhancement of atebrin fluorescence was maximal at pH 6.2 (ΔpH = 1.2), then decreased." Thus, in contrast to what was found by the present inventors, Deamer et al. teaches one of ordinary skill in the art to avoid a pH of 7-7.4 (physiologically benign) if one wants to maintain a concentration of a compound inside vesicles.

7. Cramer et al. also teaches the physical chemistry involved in using a pH gradient to load certain simple ionizable molecules (fumaric and maleic acid) into a liposome. Cramer et al. does not disclose or suggest loading liposomes with drugs; nor does Cramer et al. disclose

or suggest suspending the vesicles for administration in a bulk solution, wherein the bulk solution has a pH which is physiologically benign, as now required by independent Claims 46 and 52.

8. Like Deamer et al., Cramer et al. actually teaches away from the present invention. Specifically, Cramer et al. initially suspends liposomes in a buffer of pH 7, and then adds acid to bring the pH down (i.e., away from a physiologically benign pH). Cramer et al. notes at page 298 that "adjusting the outside pH to a lower value (4.7 compared to 5.5) results in a rapid and greater accumulation of internal fumaric acid, followed by a slow simultaneous leakage of both acids." Like Deamer et al., Cramer et al. teaches that physiological pH is to be avoided if one wants to maintain a concentration of a compound inside vesicles.

9. Kano et al. teaches the use of trisodium 8-hydroxy-1,3,6-pyrene-trisulfonate, pyranine, as a probe for monitoring the pH in the interiors of negatively charged liposomes and at the outer surface of positively charged liposomes. Kano et al. does not disclose or suggest loading liposomes with drugs; nor does Kano et al. disclose or suggest suspending the vesicles for administration in a bulk solution, wherein the bulk solution has a pH which is physiologically benign, as now required by independent Claims 46 and 52.

10. Like Deamer et al. and Cramer et al., Kano et al. actually teaches away from the present invention. Specifically, Kano et al. states at p. 298 that pH gradients are not maintained when liposomes are transferred from ph 10.00 to pH 7.12 (physiologically benign pH). Kano et al. further states that "pH gradients can be maintained in transferring liposomes from pH 7.00 to pH 2.00 or pH 9.87." Like Deamer et al. and Cramer et al., Kano et al. teaches that physiological pH is to be avoided if one wants to maintain a concentration of a compound

inside vesicles.

11. I hereby declare that all statements made herein of my own knowledge are true and that all statements were made on information and belief and are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Eric G. Mayhew, Ph.D.

15 JAN 2004
Date